REMARKS

Claims 34-38, 44-47 and 49 are canceled without prejudice or disclaimer, as drawn to the non-elected invention. Claims 31-33, 39-43 and 48 are pending. Claim 31 has been amended to correct a minor typographical error.

A new paper copy of the Sequence Listing and computer-readable form are submitted herewith. The content of the paper copy of the Sequence Listing and of the computer-readable form is the same. No new matter has been added.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. Claim Objections

Claim 31, part (b), is objected to on the basis the phrase "region position" is confusing. As suggested by the Examiner, Claim 31, part (b), has been amended to include the term "or" between terms "region" and "position." Withdrawal of the objection is respectfully requested.

II. The Rejection of Claim 32 under 35 U.S.C. 112

Claim 32 stands rejected under 35 U.S.C. 112, as allegedly indefinite on the basis that "Q153S" is unclear. The Examiner alleges that SEQ ID NO:2 shows that position 153 is occupied by an aspartic acid residue and not a glutamine residue.

Upon investigation, it has been determined that the Sequence Listing submitted in response to the Notice To Comply With Sequence Rules was erroneous. Applicants original Sequence Listing, submitted at the time of filing the application, contains a correct SEQ ID NO:2, in which position 153 is occupied by a glutamine residue. Accordingly, Applicants enclose a corrected Sequence Listing and computer-readable form herewith.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claim 31 under 35 U.S.C. 102(b)

Claim 31 is rejected under 35 U.S.C. 102(b) as allegedly anticipated by JP 07177891. The Examiner contends that JP 07177891 "teaches a mature form of an alpha-amylase that comprises an alteration in the region 98-110 of SEQ ID NO:2 wherein residues 99 and 104 are substituted, prior to this invention." This rejection is respectfully traversed.

Foremost, Applicants note that a copy of JP 07177891 was not provided with the Office Action. JP 07177891 was also not included in the Notice of References Cited. Applicants request a copy of JP 07177891. Applicants also request that this reference by included in a Notice of References Cited.

Notwithstanding the above, Applicants have obtained a copy of the abstract of JP 07177891, and have addressed the anticipation rejection based on the disclosure in the abstract. JP 07177891 is directed to variant bacterial pullulanases, i.e., from *Bacillus stearothermophilus*. Accordingly, JP 07177891 clearly does not teach the fungal-related variant alpha-amylases claimed in present application. Therefore, JP 07177891 does not anticipate the present invention.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 102(b). Applicants respectfully request reconsideration and withdrawal of the rejection.

IV. The Rejection of Claims 31-33, 39-43 and 48 under 35 U.S.C. 103

Claims 31-33, 39-43 and 48 are rejected under 35 U.S.C. 103 as allegedly obvious over Christianson et al. (U.S. Pat. No. 6,136,553) in view of Matsuura et al. (J. Biochem, 95, 697-702 (1994)) and further in view of Svendsen et al. (WO 96/23874). The Examiner contends that Christianson et al. teach (1) identifying amino acid sites in a target protein which has an effect on stability of the target protein by imputing the three dimensional coordinates into a computer, (2) generating a probe-accessible surface of the target protein, (3) identifying amino acids which make boundaries of internal cavities which have an effect on the stability of the protein, (4) mutating at least one of the sites to create a mutant target protein, and (5) expressing and isolating the mutant target protein. The Examiner states that Christianson does not disclose the alpha-amylases of the present invention as target proteins. However, the Examiner contends that Matsuura et al. disclose an amino acid sequence matching SEQ ID NO:2 of the present invention. Although neither Christianson et al. nor Matsuura et al. teach the specific alterations recited in the present claims, the Examiner contends that Svendsen et al. guides an artisan to find regions where the structure of the alpha-amylase can be mutated in order to obtain a more stable or heat resistant variant. The Examiner states that Svendsen et al. teaches that all alpha-amylase have a few conserved regions with approximately the same length and spacing and that certain alterations taught by Svendsen et al. are equivalent to alterations recited in the present claims.

This rejection is respectfully traversed. None of the cited reference teach or suggest the variant fungal-related alpha-amylases recited in the present claims. Christianson is directed to bacterial protease enzyme and methods for producing same. Matsuura et al. discloses fungal

alpha-amylases, but does not teach or suggest the alterations recited in the present claims.

Svendsen et al. is directed to bacterial-related alpha-amylase variants. The Examiner contends that Svendsen et al. teaches that all alpha-amylase have conserved regions, and that the alterations in Svendsen et al. are equivalent to the alterations in the fungal-related alpha-amylase variants of the present invention. However, the alterations of Svendsen et al. are based on the aspects of bacterial-related alpha-amylases, i.e., Termamyl-like alpha-amylases, which have very low homology and extremely low identity to the fungal related alpha-amylases of the present invention. Indeed, although Svendsen et al. teaches that there is some conservation between the bacterial and fungal alpha-amylases, Svendsen et al. specifically states that the variants it describes are "based on some striking, and not previously predicted difference between" the Termamyl-like alpha-amylase structure and both fungal and mammalian alpha-amylase. *See* Svendsen et al. at page 3, lines 6-18. Therefore, Svendsen et al., alone or in combination with Christianson et al. and Matsuura et al., clearly does not suggest the alterations in fungal-related alpha-amylases, as claimed in the present invention.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 103. Applicants respectfully request reconsideration and withdrawal of the rejection.

V. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: January 3, 2003

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Attorney Docket No.: 5835.200-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Bisgård-Frantzen et al Confirmation No: 9183

Serial No.: 09/710,339 Group Art Unit: 1651

Filed: November 9, 2000 Examiner: Momshipouri, M.

For: Fungamyl-like Alpha-Amylase Variants

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE CLAIMS:

Claim 31 has been amended as follows:

31. (Amended.) A variant of a parent Fungamyl-like alpha-amylase, comprising an alteration at one or more regions selected from the group consisting of:

Region 98-110,

Region 150-160,

Region 161-167,

Region 280-288,

Region 448-455, and

Region 468-475;

wherein (a) the alteration(s) are independently

(i) an insertion of an amino acid downstream of the amino acid which occupies the position,

(ii) a deletion of the amino acid which occupies the position, or

(iii) a substitution of the amino acid which occupies the position with a different amino acid,

(b) the variant has alpha-amylase activity and (c) each region or position corresponds to a region or position of the amino acid sequence of the parent Fungamyl-like alpha-amylase having the amino acid sequence of SEQ ID NO: 2.

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04885291 **Image available**

ENZYME-MODIFYING METHOD AND NEW NEOPULLULANASE

PUB. NO.: 07-177891 [JP 7177891 A]
PUBLISHED: July 18, 1995 (19950718)
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INTL CLASS: [6] C12N-015/09; C12N-009/44; C12N-015/09; C12R-001/07; C12N-009/44; C12R-001/19
JAPIO CLASS: 14.5 (ORGANIC CHEMISTRY -- Microorganism Industry); 14.1 (ORGANIC CHEMISTRY -- Organic Compounds)

ABSTRACT

PURPOSE: To obtain a new neopullulanase having improved hydrophobic property and increased transfer activity of a transferase either by substituting or inserting a hydrophobic group or hydrophobic amino acid to a selected site of a transferase or deleting hydrophilic amino acid from the enzyme.

CONSTITUTION: This enzyme-modifying method increases the transfer activity of a transferase such as neopullulanase originated from Bacillus stearothermophilus. The active site of the enzyme is determined e.g. by steric structure estimation by molecular modeling, a docking simulation of the enzyme with a substrate is performed by using a three-dimensional display to determine the optimum mutual configuration and the group in the selected site is replaced with a hydrophobic group or the amino acid in the selected site is replaced with a hydrophobic amino acid, or a hydrophobic group or amino acid is inserted into the selected site or a hydrophilic group or hydrophilic amino acid is depleted to increase the hydrophobia property of the selected site and obtain the new neopullulanase having improved transfer activity.
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